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Evaluation of *Tribulus terrestris* Powdered Seed as Androgenic Agent for Production of All Male Red-Belly Tilapia, *Coptodon zillii* (Gervais 1848)

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Abstract

The study investigated the effect of dietary doses of *Tribulus terrestris* on the sex reversal and production of *Coptodon zillii* fry. A total of two hundred and seventy (270) three days old apparent healthy fry of *C. zillii* (mean weight 0.03 ± 0.0g) were randomly selected and distributed into 18 glass tanks (70x45x45 cm^3) at 15 fish per tank representing five treatments in triplicate to contain *T. terrestris* at different inclusion levels of 0.00 (negative control), 10.0, 15.0, 20.0 and 25.0g/kg *T. terrestris seeds powder* basal diet and labelled (T1-T5). A reference diet contain 0.06 g/kg of basal diet of 17α methyltestosterone (MT) was also formulated as positive control and labelled (T6). At the end of the experiment, final growth weights were recorded and the carcass composition determined. Gonad squashing method involving aceto-carmine staining was used to determine the sex of the fish. Results indicated that dietary treatment with powdered T. terrestris seeds and MT at different concentrations showed no significant difference (P>0.05) in survival percentage compared to that in control group, and growth parameters were better in fish fed *T. terrestris and* MT treated groups than in the fish fed T1. The highest weight gain was recorded in fish in T4 while the least value was observed in fish fed control diet. All fish fed diet containing *T. terrestris and* MT treated groups had significantly higher male proportion than untreated group (36.2%). The percentage of male (78.5%) population in *T. terrestris* treatments in this study was significantly lower than MT treated group (92.1%) and significantly higher than untreated group. Carcass protein decreased with increasing levels of *T. terrestris* in the diet. The study indicated that *T. terrestris* seed powder might be used as a potential alternative to synthetic steroid for production of all male tilapia population.

Keywords: Sex reversal, *Tribulus terrestris* and *Coptodon zillii.*

Introduction

Tilapia production is yet to reach full aquaculture potential because of the problems of precocious maturity and uncontrolled reproduction which often results in the overpopulation of production ponds with young and stunted fish (<100 g) that are bony and thus with poor market value (Fagbenro and Sydenham, 1997). Two effective methods that have been used to control such undesirable tilapia population are mixed predator-tilapia stocking and mono-sex culture. Male tilapias grow faster than females (Myers, *et al.,* 1995; Tariq Ezaz, *et al.,* 2004). Tilapia fish can be

masculinized by direct synthetic hormonal treatment that is efficient and straight forward (Pandian and Sheela, 1995; Gale, *et al.,* 1999). A more important disadvantage of synthetic hormone is the fear of residues in fish and products. There is evidence that chemicals naturally occurring in plants could provide a useful source of masculinizing agents (Lohiya *et al.,* 1999).

Phytochemicals have also been reported to block biosynthesis as well as action of estrogen by acting as aromatase inhibitors and antagonists to nuclear estrogen receptor in gonad germ cells (Rempel and Schlenk, 2008) and hence may be considered as potential means for inducing sex reversal in fish. The herb, *T. terrestris* has been reported to raise testosterone levels (Bucci, 2000) and to induce sex reversal in fish while administered through immersion technique. The plant extract has also been found to stimulate growth in *Poecilia reticulata* and *Cichlasoma nigrofasciatum* (Çek *et al.*, 2007a; Çek *et al.*, 2007b). Also, it has been found to be effective for production of monosex in *Poecilia latipinna* population (Kavitha and Subramanian, 2011). The alternatives that can be considered to reduce the use of synthetic steroid hormone for sex reversal of tilapia is the (*T. terrestris* seed powder) which are available at local markets in the country. The main objective of this study was to determine the effect of *T. terrestris* seed powder in masculinization of *C. zillii* by oral administration.

Materials and Methods

Collection of plant samples

Tribulus terrestris seeds were procured from a local plant market at Bodija Ibadan, Oyo State, Nigeria. It was identified and authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. The seeds were washed in distilled water and dried at room temperature to prevent loss of volatile compounds. The seeds were ground into fine powder using an electric blender (Model ES 242) and stored at 4°C in a container until used.

Experimental fish

Three days old mixed sex fry of *Coptodon. zillii* were collected from the Fish Hatchery of the Federal University of Technology Akure. Fry were transferred into glass tanks of dimension (70cm x 45cm x 45cm) and acclimatized for 24 hrs without feeding before the commencement of feeding with experimental diets in the laboratory.

Preparation of experimental diets

Six iso-nitrogenous diets (35% CP) were formulated for *C. zillii* to contain *T. terrestris* at different inclusion levels of 0.0 g (negative control), 10, 15, 20 and 25g/kg *T. terrestris* seeds powder basal diet and labelled (T1-T5). A reference diet contain 0.06 g/kg of basal diet of 17α methyltestosterone (MT) was also formulated as positive control and labelled (T6). Diets were fan dried under room temperature for 48 hours packed in polyethylene bags, sealed, and marked according to treatments and stored at 4°C before use. The diets were then blended into powdery form before feeding to the fry.

Experimental design

The experimental design was a completely randomized design. A total of two hundred and seventy (270) *C. zillii* with mean weight of 0.03 ± 0.00g were randomly selected and distributed into 18 glass tanks (70 litre) each measuring (70cm x 45cm x 45cm) at the rate of 15 fish per tank representing six treatments and three replicates.

Feeding of experimental fish

The fry were fed with experimental diets three times daily between 08:00 and 09:00, 12:00 and 01:00 and 16:00 and 17:00h GMT to apparent satiation for 90 days. 75% of water in each tank was changed daily in the morning to avoid water quality deterioration due to decomposition of feces of the fry. In addition, the faecal wastes were removed from the tanks by siphoning at 08:00 and 4:00 hours daily before feeding. Water quality parameters (temperature, dissolved oxygen and pH) were determined twice a week. Calculation of the growth performance was according to Takeuchi (1988) and Tacon (1990). The plasma testosterone level was according to the methods described by Neychev and Mitev (2005). Sexing of the juvenile fish was done by the standard acetocarmine squash technique of gonads as described by Guerrero and Shelton (1974) which

was validated for *O. niloticus,* Wassermann and Afonso (2002).

Data collection and analysis

All data were expressed in terms of mean±standared error. Treatment effects on different parameters were analysed by one-way analysis of variance (ANOVA) as described by Steel and Torrie (1980). Duncan's New Multiple Range Test (Duncan, 1955) was used to separate differences among the means using the computer software SPSS (Statistical Package for Social Science Version 20) and (Microsoft office Excel programme 2010). Proximate analysis of the diets and carcass composition of the fish was carried out according to AOAC (1990) methods.

Table 1: Proximate composition of *T. terrestris* seed

Parameters (%)	Proximate
Moisture	7.60
Ash	9.84
Protein	11.3
Fibre	35.2
Lipid	12.9
NFE	23.16

KEY; N F E= Nitrogen free extract

Results and Discussion

The nutrient composition of dried *T. terrestris* seed is presented in Table 1. The result showed that *T. terrestris* has a low moisture content; which could be attributed to the period of sampling which was about onset of dry season, a season characterized by intensive sunlight and dryness. The lipid content is high enough to supply fats and oil to the diets. It is also high in non-protein value that can be advantageous as energy supply.

Table 2 shows the gross and proximate composition of diets fed to the experimental fish. The proximate composition of the diets revealed that dietary *T. terrestris* increased the ash, fibre and lipid contents of the diets.

The observed protein content ranged from 35.0 to 35.7%. El-Sayed and Gaber (2005) reported that the dietary protein requirements of several species of tilapia have been estimated to range from 20 to 56%. Therefore, protein content of the test diet used in this study was within the suitable range for tilapia culture.

Table 2: The gross and proximate composition of the experimental diets (g/100g)

Ingredients	T1	T2	T3	T4	T5	T6
Fish meal	16.8	16.8	16.8	16.8	16.8	16.8
Soybean meal	30.0	30.0	30.0	30.0	30.0	30.0
Groundnut cake	22.1	22.1	22.1	22.1	22.1	22.1
Yellow maize	18.6	18.6	18.6	18.6	18.6	18.6
Methionine	1.0	1.0	1.0	1.0	1.0	1.0
Lysine	1.0	1.0	1.0	1.0	1.0	1.0
Fish oil	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin premix**	3.5	3.5	3.5	3.5	3.5	3.5
Starch	2.0	2.0	2.0	2.0	2.0	2.0
Powdered seeds of *T. terrestris* (g/kg)	0.0	10.0	15.0	20.0	25.0	-
MT (g/kg)	-	-	-	-	-	0.06
Proximate composition						
Moisture	8.05	7.94	8.26	8.21	8.19	7.59
Ash	5.77	6.15	6.19	6.22	6.23	6.20
Protein	35.5	35.3	35.1	35.0	34.8	35.7
Fibre	4.13	5.08	5.12	5.24	5.32	4.18
Lipid	8.82	9.13	9.17	9.20	9.26	8.11
Nitrogen free extract	37.7	36.4	36.2	36.1	36.2	38.2
Gross Energy	439.0	435.3	433.6	433.2	432.9	435.4

The growth performance indices of *C. zillii* in the experiment are presented in Table 3. At the end of the experiment, the highest weight gain was recorded in fish fed diet 4 while the least value was observed in fish fed diet 1. Percentage survival in diet 1 was similar to those observed in the *T. terrestris* and MT treated groups, where no significant differences were noted ($p>0.05$). The use of *T. terrestris* at high inclusion levels (25 g/kg) in this study led to a reduction in growth of the fish. Similarly, Appler (1985) reported that there was reduced growth in *Tilapia zillii* with increasing levels of freshwater algae *Hydrodictyon reticulatum* at 5% inclusion level. The MT-treated diet in this study contained a dose of 0.06 g/kg which is high enough to promote growth aside from sex-reversing the fry. These results are in line with the findings regarding anabolic effect of MT in fish and all male culture of ornamental fish by Mousavi-Sabet (2011) who observed faster growth rate in hormone (MT) treated *Cichlasoma nigrofasciatum.* Some authors have reported similar observations for other fish species, treated with synthetic androgen (Sparks *et al.,* 2003) on *O. mossambicus,* (Deborah, 2009) on *Ictalurus punctatus*, (Henry, *et al.,* 2002) and on *Hippoglossus hippoglossus.* Ridha and Lone (2008) reported that different dose rates of MT significantly increased the growth of *O. niloticus* fry than the control.

The percentage of males (36.2%) in the untreated group was significantly lower ($p<0.05$) than the ones in the *T. terrestris* and MT treated groups (Table 4). Results of the present study demonstrated that *T. terrestris* and MT showed more potential for sex reversed into male than with the control group, but the highest male population for *C. zillii* was recorded in MT treated group. The population of male fish increased with increasing levels of *T. terrestris* in the diets. This result agreed with the report of (Mengumphan *et al.,* 2006) in which they reported increase in percentage of males in Ghana strain of *O. niloticus* fed dried and ground roots of Red Kwao Kreua (*Butea superba*). Percentage of male population obtained in this study (78.5%) for fish treated with *T. terrestris* was lower than that reported by (Francis *et al.,* 2002) on Nile tilapia fry fed diets supplemented with *Quillaja saponaria,* but higher than (65.0%) reported by Ampofo-Yeboah (2013) in O*reochromis mozambicus* fed pawpaw seed meal at 20 g/kg. The proportion of males (92.1%) recorded in MT treated group in this study was lower than that reported by Green and Teichert-Coddington (2000) where 96.8% males were recorded with 60 mgMT/kg for 28 days in *O. niloticus* and Das *et al*. (2011) with (96%) male population in *O. niloticus* fed 60 mgMT/kg for 45 days.

Table 3: Growth performance and nutrient utilisation of *C. zilli* fed the experimental diets

Parameters	T1	T2	T3	T4	T5	T6
IW (g)	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}
FW (g)	2.55 ± 0.10^{a}	2.70 ± 0.12^{ab}	2.89 ± 0.25^{b}	3.27 ± 0.25^{c}	3.01 ± 0.02^{b}	2.74 ± 0.02^{b}
WG (g)	2.52 ± 0.10^{a}	2.67 ± 0.12^{ab}	2.86 ± 0.25^{b}	3.24 ± 0.25^{c}	2.98 ± 0.12^{b}	2.71 ± 0.02^{b}
SGR(%/day)	4.98 ± 0.05^{a}	5.00 ± 0.06^{ab}	5.08 ± 0.08^{ab}	5.21 ± 0.11^{c}	5.12 ± 0.56^{b}	5.02 ± 0.01^{ab}
FI (g)	3.97 ± 0.07^{a}	4.02 ± 0.05^{b}	4.07 ± 0.12^{b}	4.41 ± 0.05^{d}	4.29 ± 0.01^{c}	4.13 ± 0.05^{c}
FCR (g)	1.58 ± 0.08^{d}	1.51 ± 0.06^{c}	1.44 ± 0.09^{b}	1.38 ± 0.11^{a}	1.44 ± 0.06^{b}	1.52 ± 0.02^{c}
Survival (%)	80.7 ± 2.23^{a}	82.2 ± 5.87^{a}	77.7 ± 3.38^{a}	75.0 ± 9.67^{a}	84.4 ± 2.23^{a}	82.2 ± 5.87^{a}

Means in same row with different superscripts are significantly different at ($P<0.05$)

Table 4: Effect of *T. terrestris* on the sex ratio of *C. zillii* fed the experimental diets

Treatments	**Male (%)**	**Female (%)**
Control (0 g/kg	36.2 ± 2.76^{a}	63.8 ± 2.76^{d}
10.0 g/kg	57.6 ± 0.93^{b}	42.4 ± 0.93^{c}
15.0 g/kg	72.8 ± 0.23^{c}	27.2 ± 0.23^{b}
20.0 g/kg	77.6 ± 0.32^{cd}	22.4 ± 0.32^{ab}
25.0 g/kg	78.5 ± 1.67^{d}	21.5 ± 2.77^{ab}
0.06 g/kg	92.1 ± 0.83^{e}	7.90 ± 0.83^{a}

Means in same row with different superscripts are significantly different at ($P<0.05$)

Table 5: Carcass composition of *C. zillii* fed the experimental diets (dry)

Parameters (%)	T1	T2	T3	T4	T5	T6
Moisture	6.48±0.03^{a}	7.58±0.07^{c}	7.24±0.27^{bc}	7.15±0.01^{b}	7.20±0.01^{bc}	9.23±0.15^{d}
Ash	5.93±0.09^{c}	4.37±0.03^{a}	4.43±0.09^{ab}	5.08±0.00^{b}	4.41±0.07^{a}	6.91±0.03^{d}
Crude protein	57.4±0.13^{c}	50.6±0.03^{b}	46.2±0.19^{ab}	46.1±0.33^{ab}	45.2±0.03^{a}	57.5±0.31^{c}
Crude lipid	18.3±1.00^{b}	18.9±0.17^{b}	20.4±0.18^{c}	20.6±0.41^{c}	21.3±0.25^{d}	17.3±0.38^{a}
NFE	11.89±0.15^{b}	18.55±0.35^{bc}	21.73±0.06^{c}	21.07±0.14^{c}	21.89±0.23^{c}	9.06±0.66^{a}

Means in the same row with different letters are significantly different at P<0.05

Table 5 reveals that there were significant differences (p<0.05) in the body composition of the fish. Compared to diet 1 (control), protein levels of fish body decreased after feeding the fish with *T. terrestris* (P<0.05). Fish fed on *T. terrestris* had a significantly (p<0.05) higher lipid content than fish fed diet 1 and MT treated group. The highest lipid values obtained in fish fed diet 5 may be due to the highest lipid content (9.26%) observed in diet. Goda *et al.,* (2007) reported similar result for *O. niloticus* and *Sarotherodon galilaeus* fingerlings fed plant-based protein diets. The authors found increase in lipid content and this finding is not contradictory with the present results. A higher lipid composition was also reported in *O. niloticus* fed a higher dietary carbohydrate regime **(**Anderson *et al.,* 1984) and Francis *et al*. (2001) observed that 300 mg/kg *Quillaja saponaria* saponin in the diet of *O. niloticus* increased the lipid content. Fish fed MT had the highest protein content when compared to other groups. This is supported by the finding of Pechsiri and Yakupitiyage (2005) who reported that *O. niloticus* fed MT treated group had significantly higher protein content than the control group.

Conclusion

The results emanating from this study indicate that *T. terrestris* can be used as an alternative method to produce all-male tilapia. However, the highest percentage of males 78.5% produced by the plant material was found to be well below the expected 100% male population. Thus, further studies are required to establish an ideal treatment regime for production of all-male tilapia population using the plant material and to provide conclusive evidence regarding the efficacy to be used as a sex-reversal agent in tilapia culture.

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